EMBRYOLOGICAL EVIDENCE SUPPORTS THE TRANSFER OF *LEITNERIA FLORIDANA* TO THE FAMILY SIMAROUBACEAE[1]

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ABSTRACT

Leitneria floridana Chapm., a shrub indigenous to North America, was long considered to be the sole member of the family Leitneriaceae, with uncertain affinities in and outside the class Amentiferae. Recent molecular analyses, however, show that the species is closely related to the genera *Brucea* J. F. Mill., *Soulamea* Lam., and *Amaroria* A. Gray in the family Simaroubaceae. Here, I report embryological features of *L. floridana* and provide anatomical corroboration for the molecular simaroubacean affinities of this species. I compared its embryology with those of the families Simaroubaceae, Meliaceae, and Rutaceae, which form a monophyletic clade in the order Sapindales. Comparisons showed that although *Leitneria* Chapm. resembled all three families embryologically, it fits best within the Simaroubaceae through possession of an unspecialized chalaza, straight and exarillate seeds, a non-multiplicative testa, a non-crystalliferous endotesta, and absence of a fibrous exotegmen. Within the Simaroubaceae, *Leitneria* also shares with *Brucea* the synapomorphies of a micropyle formed by an elongate, irregularly folded inner integument and a persistent tegmen composed only of endotegmen. Members of the Simaroubaceae are still poorly known embryologically, but available evidence supports the close affinity of *Leitneria* and *Brucea*.

Key words: *Brucea*, embryology, *Leitneria*, Sapindales, seed structure, Simaroubaceae.

Leitneria floridana Chapm. (Florida corkwood) is a dioecious shrub found in low, wet areas of the southeastern and south-central United States and is the only species of the genus with characteristic erect catkins (Channell & Wood, 1962; Bogle, 1997). The genus was first placed in the family Myricaceae (Chapman, 1860: 427), and thereafter, because of its uncertain affinities, was reassigned to become the sole member of the family Leitneriaceae, placed variously within the orders in and outside of the class Amentiferae: Leitneriales (e.g., Bentham & Hooker, 1880; Hjelmquist, 1948; Channell & Wood, 1962; Melchior, 1964; Cronquist, 1981; Bogle, 1997), Sapindales (Dahlgren, 1980), Rutales (including Sapindales) (Thorne, 1992), or Leitneriales near Sapindales (Takhtajan, 1997), or the superorder Rutanae (Takhtajan, 2009). Affinities with the family Simaroubaceae were first suggested by serological studies (Petersen & Fairbrothers, 1983, 1985). Petersen and Fairbrothers (1983) obtained antigens from *Leitneria* Chapm. pollen protein extracts and found that the strongest reactivity of *Leitneria* antigen was with *Ailanthus* Desf. and *Picrasma* Blume, both genera in the Simaroubaceae. Thereafter, molecular analyses based on *rbcL* sequences (Chase et al., 1993; Fernando et al., 1995; Gadek et al., 1996) consistently showed that *Leitneria* is best positioned in the Simaroubaceae, a family forming a clade inclusive of the families Rutaceae and Meliaceae in the order Sapindales. The Angiosperm Phylogeny Group (1998, 2003, 2009) accepted *Leitneria* as a member of the Simaroubaceae (see also Thorne & Reveal, 2007).

Most recent molecular analyses based on sequences from three plastid genes (*rbcL*, *atpB*, and *matK*) and a nuclear gene (*phyC*) show that *Leitneria* is sister to a clade comprising the genera *Brucea* J. F. Mill., *Soulamea* Lam., and *Amaroria* A. Gray within the Simaroubaceae (Clayton et al., 2007). This is a striking result and an example of the way in which molecular analyses have successfully resolved long-standing systematic uncertainties. Our concern was to find morphological synapomorphies likely to corroborate the sister-group relationship between *Leitneria* and the clade comprising *Brucea*, *Soulamea*, and *Amaroria*. Clayton et al. (2007: 1334) opined that morphological synapomorphies for these genera "may be difficult to detect due to the dramatic floral divergence of *Leitneria*."

I have long been interested in the relationships of *Leitneria* and have studied the genus embryologically for comparison with purportedly related taxa. More than 50 embryological characters are relevant to the development of the anther, ovule, and seed,

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[1] I am grateful to Peter H. Raven, Peter C. Hoch, and George Yatskievych for their assistance with collecting materials used in this study. George Yatskievych took photographs of inflorescences used in the figures of this paper.

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doi: 10.3417/2009073

ANN. MISSOURI BOT. GARD. 98: 277–293. PUBLISHED ON 15 JULY 2011.

which have often provided evidence of relationships among taxa ranging from species to families (Tobe, 1989). About 100 years ago, some of the embryological characters of *Leitneria* were described by Pfeiffer (1912) for comparison with members of the Amentiferae; plants used for this study were cultivated at the Missouri Botanical Garden (MO). However, most of the characters were not documented with the precision expected in modern works, and many other aspects of embryology are as yet undescribed. We now need exact information on the largest suite of embryological characters for critical comparison with other members of the Simaroubaceae. In this study, I confirm or correct Pfeiffer's (1912) interpretations and document almost all of the embryological features of *L. floridana*. These are compared with characters of members of the Simaroubaceae, Meliaceae, and Rutaceae. I show the embryological relatedness of *Leitneria* within the Simaroubaceae, particularly with the genus *Brucea* and its relatives.

MATERIALS AND METHODS

Staminate flower buds of *Leitneria floridana* at various stages of development were collected from trees cultivated at the Missouri Botanical Garden in 2000, 2001, and 2007 (voucher: U.S.A., Missouri, St. Louis City, Missouri Botanical Garden, cultivated, perhaps originated in Butler Co., MO, *W. G. D'Arcy 3653* [MO 2426777]), and pistillate flower buds and fruits at various stages of development from trees in Butler County, Missouri, in 2000 and 2001 (voucher: U.S.A., Missouri, Butler Co., edge of swamp, S side of Neelyville Jct., *W. G. D'Arcy et al. 4557* [MO 2155947]). Samples were fixed in five parts stock formalin/five parts glacial acetic acid/90 parts 50% ethanol (FAA). Flower buds and seeds were dehydrated through an ethanol series and then embedded in Technovit 7100 (Kulzer, Wehrheim, Germany) for microtoming. Serial resin sections cut at a thickness of 5–7 μm were stained with Heidenhain's hematoxylin and mounted in Entellan (Merck, Darmstadt, Germany). The slides' permanent mounts are housed at the Department of Botany of Kyoto University.

RESULTS

ANTHERS AND MICROSPORES

The staminate inflorescence is composed of about 40 to 50 flowers (or partial inflorescences) that are spirally arranged on the fertile axis (Fig. 1A). Each staminate flower, which we interpret as a 3-flowered cymule (Abbe & Earle, 1940; Abbe, 1974), bears about 10 stamens and is subtended by the primary bract (Fig. 1B). The anther is tetrasporangiate (Fig. 1B, F). Prior to maturation the anther wall comprises five or six cell layers, namely an epidermis, an endothecium, two or three middle layers, and a tapetum (Fig. 1C). The anther epidermis is persistent but collapsed in places. How the middle layers form remains uncertain. The tapetum is glandular (Fig. 1D, E) and its cells are usually multinucleate, with three to five nuclei each (Fig. 1E). Nuclei in tapetal cells often appear to be fused. During maturation, the middle layers degenerate and the epidermal cells become somewhat enlarged. Cells of the endothecium become enlarged and develop fibrous thickenings (Fig. 1D, F, G). Anther dehiscence takes place along longitudinal slits, with each slit common to two thecal microsporangia (Fig. 1G).

In the absence of suitable study materials, I was unable to ascertain whether microspore mother cell meiosis is accompanied by simultaneous or successive cytokinesis. Microspore tetrads are predominantly tetrahedral (Fig. 1D). Mature pollen grains are 2-celled (Fig. 1H).

MEGAGAMETOPHYTE AND NUCELLUS

The pistillate inflorescence is composed of 12 to 15 flowers arranged spirally on the fertile axis (Fig. 2A). Each flower has a single pistil with an elongate style and a superior ovary, bearing a fertile ovule on the upper side and a rudimentary ovule on the lower side in an ovarian locule (Fig. 2B). The fertile ovule is epitropous with a micropyle pointing upward; it is crassinucellate. In the absence of the youngest flower buds, I was unable to determine the number of archesporial cells. Each of the youngest buds available (collected 4 March 2000) had a single megasporocyte differentiated beneath a parietal tissue that was two cell layers in thickness (Fig. 2C). The parietal cells further divide periclinally, resulting in a thick parietal tissue (more than 10 cells thick) by the time the female gametophyte develops (Fig. 2F). The megasporocyte undergoes meiosis, resulting successively in a dyad (Fig. 2D), and then a linear tetrad of megaspores (Fig. 2E), as reported by Pfeiffer (1912). In the megaspore tetrad, the chalazal megaspore is functional and the three micropylar megaspores degenerate (Fig. 2E, F). The functional megaspore develops sequentially into a 2- (Fig. 2G), 4- (Fig. 2H), and 8-nucleate female gametophyte (Fig. 2I), as reported by Pfeiffer (1912). Therefore, the mode of female gametophyte development corresponds to the Polygonum type (for diverse modes of female gametophyte development in

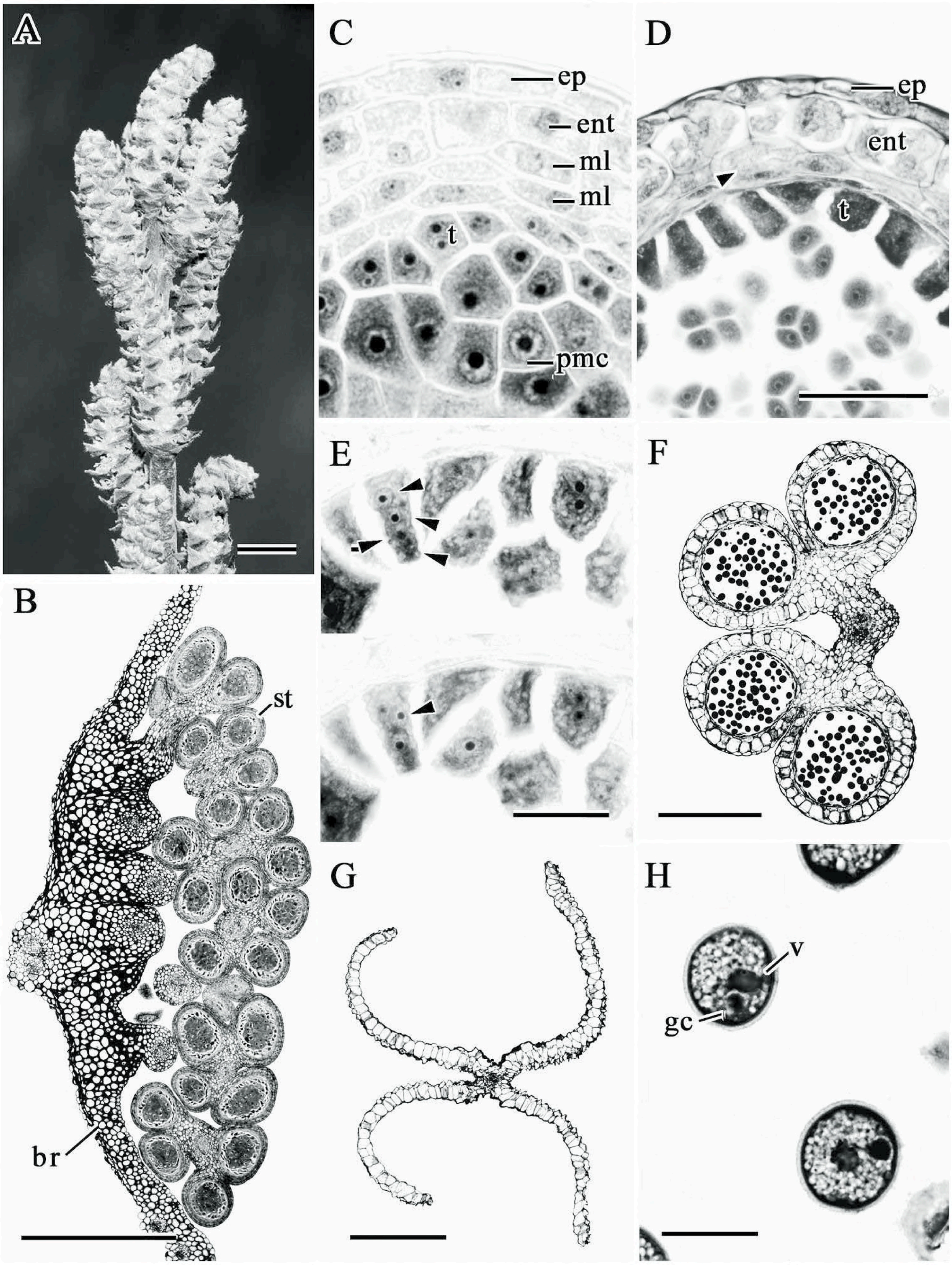

Figure 1. *Leitneria floridana*, development of anthers and microspores. —A. Staminate inflorescence. —B. Transverse section of a staminate flower (or partial inflorescence). —C. Transverse section of a young anther. —D. Transverse section of an older anther, showing a developing anther wall. Arrowhead indicates the degenerating middle layers. —E. Transverse section of an older anther, showing multinucleate glandular tapetal cells at two focal planes. Five nuclei in a single cell are indicated by arrowheads. —F. Transverse section of a nearly mature anther. —G. A mature anther dehisced along longitudinal slits. —H. Mature pollen. Abbreviations: br, bract; ent, endothecium; ep, epidermis; gc, generative cell; ml, middle layer; pmc, microspore mother cell; st, stamen; t, tapetal cell; v, nucleus of a vegetative pollen cell. Scale bars: A = 1 cm; B = 500 μm; F, G = 300 μm; D = 50 μm; C, E, H = 20 μm. A–H taken from *W. G. D'Arcy 3653* (MO).

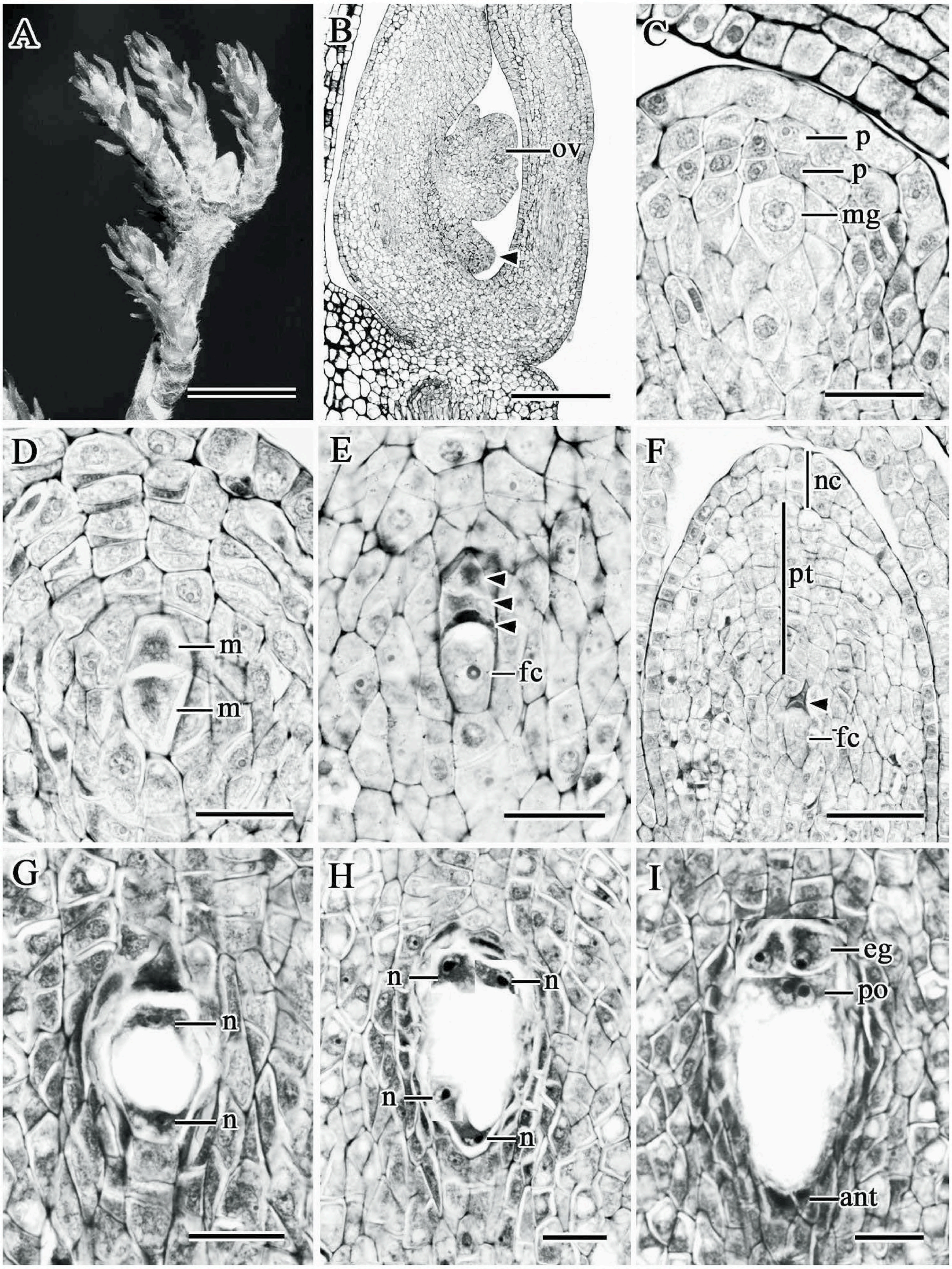

Figure 2. *Leitneria floridana*, development of ovules and female gametophytes. —A. Pistillate inflorescence. —B. Longitudinal section of a young pistil, showing one fertile ovule above and a lower rudimentary ovule (to left of arrowhead) in the locule. C–I. Longitudinal section of ovules in various stages of development. —C. Young ovule with a megasporocyte. —D. Ovule with a dyad of megaspores. Each cell is at meiotic metaphase II, with the chromatids equatorially aligned. —E. Ovule with a tetrad of megaspores; the upper three spores (to left of arrowheads) are degenerating. —F. Ovule with a uninucleate female gametophyte. Note a thick nucellar cap and thick parietal tissue have formed. —G. Binucleate female gametophyte or embryo sac. —H. Four-nucleate stage of the female gametophyte visualized by combining four consecutive serial sections. —I. Mature female gametophyte, visualized by combining two consecutive serial sections. One cell of an egg apparatus appears in the following section. Abbreviations: ant, antipodal cell; eg, egg cell; fc, functioning megaspore; m, megaspore; mg, megasporocyte; n, nucleus of female gametophyte; nc, nucellar cap; ov, ovule; p, parietal cell; po, polar nuclei; pt, parietal tissue. Scale bars: A = 1 cm; B = 200 µm; F, I = 50 µm; C–E, G, H = 20 µm. A–I taken from *D'Arcy, Porter & Demeree 4557* (MO).

angiosperms, see Stuessy, 2009: 216). The organized mature female gametophyte is ellipsoidal, comprising an egg cell, two synergids, two polar nuclei, and three antipodal cells. The three antipodal cells are ephemeral (Fig. 2I) and disappear soon after fertilization. During megasporogenesis, the apical dermal cells of the nucellus divide periclinally to form a nucellar cap that is three cell layers thick (Fig. 2F), as reported by Pfeiffer (1912).

At maturity, the ovule is hemitropous with the micropyle oriented at right angles to the chalaza (Fig. 3D). The nucellar tissue is very thick around the mature female gametophyte (Fig. 3D). At this stage, the parietal tissue lying above the mature female gametophyte is up to 15 cell layers thick (Fig. 3D). It thickens further and persists through post-fertilization stages without being crushed by the enlarging female gametophyte (Fig. 4A, D, E); it may also persist in mature seeds. No set sequence of further development occurs in the chalaza, which may result, for example, in pachychalazy. A hypostase does not differentiate, even in post-fertilization stages. No conspicuous obturator is formed.

INTEGUMENTS

The ovule is bitegmic (Fig. 3A–D). The inner integument is initiated first and the outer integument much later and is always shorter than the inner (Fig. 3A–C). On the antiraphal side, both the inner and outer integuments are initially two cells thick (Fig. 3A), but through anticlinal divisions of inner epidermal cells, soon become three cells thick along most of their lengths (Fig. 3B–E). In contrast, on the raphal side, the inner integument is thicker, dividing to four cells thick (Fig. 3B). Neither the inner nor the outer integument is multiplicative, so that their respective thicknesses do not change throughout development. No vascular supply was found in either the inner or outer integument.

The micropyle is formed by the inner integument alone when the female gametophyte is in the early 2- to 4-nucleate stage. This integument grows beyond the apex of the nucellus within the confined space of the locule, so that its tip is extremely elongated and irregularly folded (Fig. 3D).

ENDOSPERM AND EMBRYO

Although I did not confirm pollen tube passage through the micropyle, there is no doubt that the pollen tube grows from the nucellar apex to the female gametophyte through the thick parietal tissue (Fig. 4A). In post-fertilization stages, cell divisions continue in the nucellus, so that a thick nucellar tissue is formed on the chalazal side (Fig. 4B). A circumscribed nucellar tissue eventually disappears as the seed grows, but a degenerating fragment may remain in mature seeds (Fig. 4I).

Endosperm formation is of the nuclear type, and free nuclei are observed in the female gametophyte during early embryogenesis (Fig. 4C), as reported by Pfeiffer (1912). By the early stages of embryogenesis, the endosperm is cellularized centripetally (Fig. 4D). In mature seeds, the 10- to 30-cell-layered endosperm remains located outside the large embryo (Fig. 4H).

I did not examine embryogenesis in detail in this study, but fragmentary data on early and later embryogenesis indicate that the process progresses normally from proembryos through fully developed embryos (Fig. 4C–E). The suspensor is not especially large (Fig. 4E). The embryo in a mature seed is straight, large, and dicotyledonous (Fig. 4F).

SEED AND SEED COAT

Fruits are straight drupes with smooth surfaces that are about 27–30 mm long and 5–8 mm wide, each containing a single seed (Fig. 4F, upper right). The seed is enclosed by a hard endocarp composed of sclerotic cells (Fig. 4F). When the seed is still young, the coat comprises a 3- or 4-cell-layered tegmen (i.e., a developed inner integument) and a 4- or 5-cell-layered testa (i.e., a developed outer integument) (Fig. 4G). Among cells of the tegmen, those of the innermost layer, that is, the endotegmen, stain darkly, while cells of the outer layers are not specialized (Fig. 4G). Within the layers of the testa, the outermost layer cells are somewhat enlarged, while those of the other layers are unspecialized (Fig. 4G).

Mature seeds are straight and exarillate. The testa is slightly multiplicative and becomes five to 11 cell layers thick (Fig. 4H, I). Although most of the cell layers of the testa persist, those of the tegmen degenerate (except for the innermost layer). Thus, the mature seed coat comprises a 5- to 11-cell-layered testa and a 1-cell-layered tegmen (i.e., an endotegmen) (Fig. 4I). Cells of the exotesta are variously enlarged and contain scattered granular pigments. Cells of the mesotesta and endotesta are unspecialized, although Pfeiffer (1912: 198) observed pitted walls in all of them. Cells of the endotegmen have slightly thickened walls, as observed by Pfeiffer (1912). The seed coat may be categorized as testal (for seed coat terminology, see Corner, 1976; Schmid, 1986).

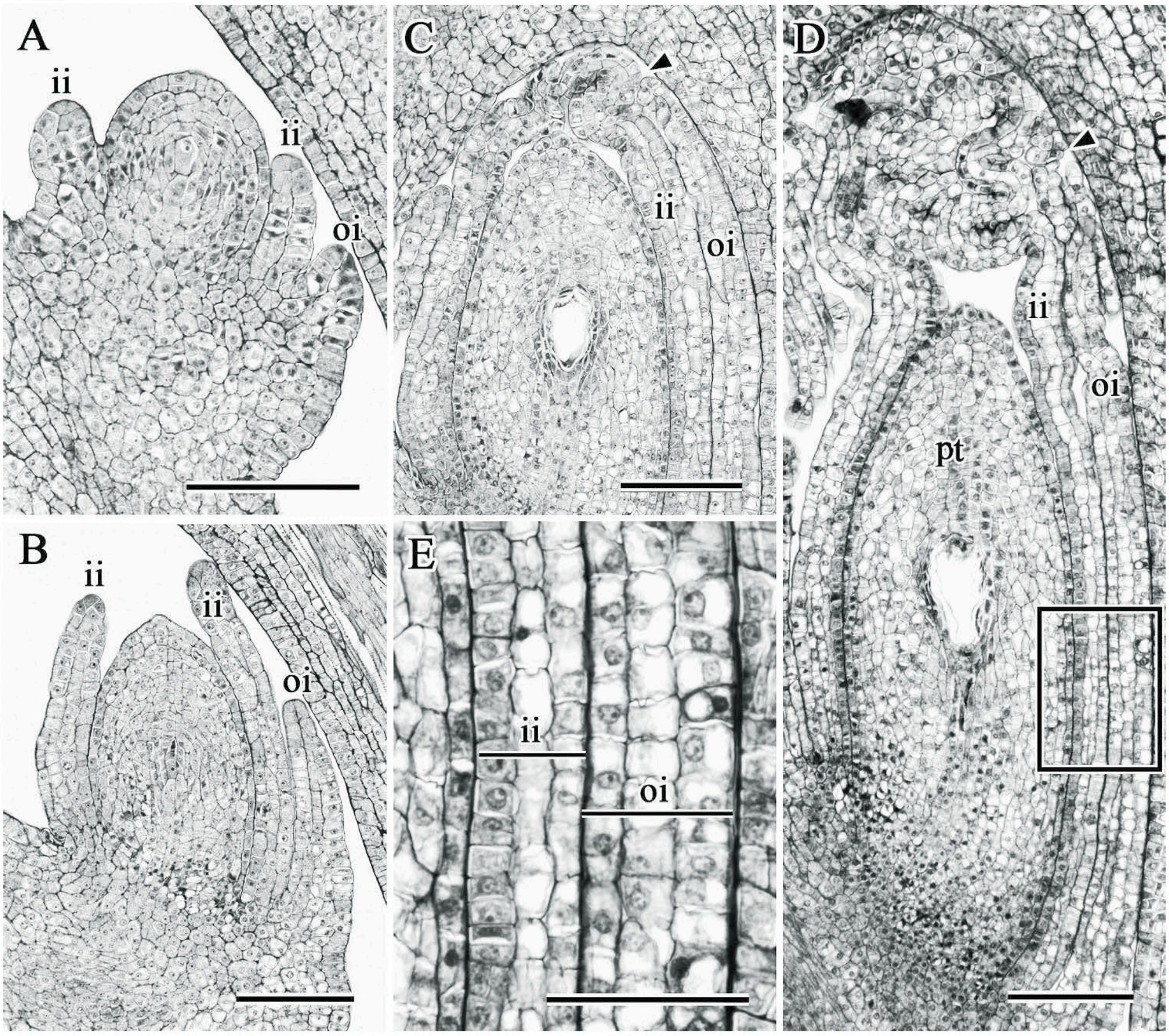

Figure 3. *Leitneria floridana*, development of integuments and micropyle. —A–D. Longitudinal sections of young and mature ovules showing development of the integuments as the ovules develop in the sequence from A to D. All sections were cut through the raphe to the antiraphe. Note that the outer integument is always shorter than the inner and that the micropyle is formed by the inner integument alone, which develops beyond the nucellar apex and becomes irregularly folded in an ovarian locule (D). Arrowheads in C and D indicate the position of the tip of the outer integument. —E. Magnified view of the ovule enclosed by a rectangle in D. Abbreviations: ii, inner integument; oi, outer integument; pt, parietal tissue. Scale bars: A–D = 100 μm; E = 50 μm. A–E taken from *D'Arcy, Porter & Demeree 4557* (MO).

Discussion

SUMMARY OF EMBRYOLOGICAL FEATURES

Prior to my analysis, information on the development of the anther, ovule, and seed was fragmentary, although a few embryological characters were described by Pfeiffer (1912). My work provides most of the missing information and updates current concepts for several characters. Here, I summarize what is now known about the embryology of *Leitneria floridana* (see also Table 1).

The anther in *Leitneria floridana* is tetrasporangiate, with the anther wall five or six cell layers thick and of uncertain formation. The anther epidermis is persistent but partially collapsed; the endothecium is fibrous; two or three middle layers are crushed; the tapetum is glandular, its cells usually multinucleate and containing more than three nuclei. Cytokinesis in the microsporocyte is uncertain; microspore tetrads are predominantly tetrahedral, and pollen grains are 2-celled when shed.

The ovule in *Leitneria floridana* is hemitropous and crassinucellate, and the number of archesporial cells is uncertain, but the youngest ovules observed have a single megasporocyte beneath a 2-cell-layered parietal tissue, with the parietal tissue thickening to 15 cell layers by the mature female gametophyte stage. The megasporocyte undergoes meiosis, and the megaspore tetrads are linear. The chalazal megaspore is functional, developing into an 8-nucleate Polygonum-type female gametophyte, and the antipodal cells are ephemeral. Apical nucellar epidermal cells are periclinally divided, forming a 3-cell-layered

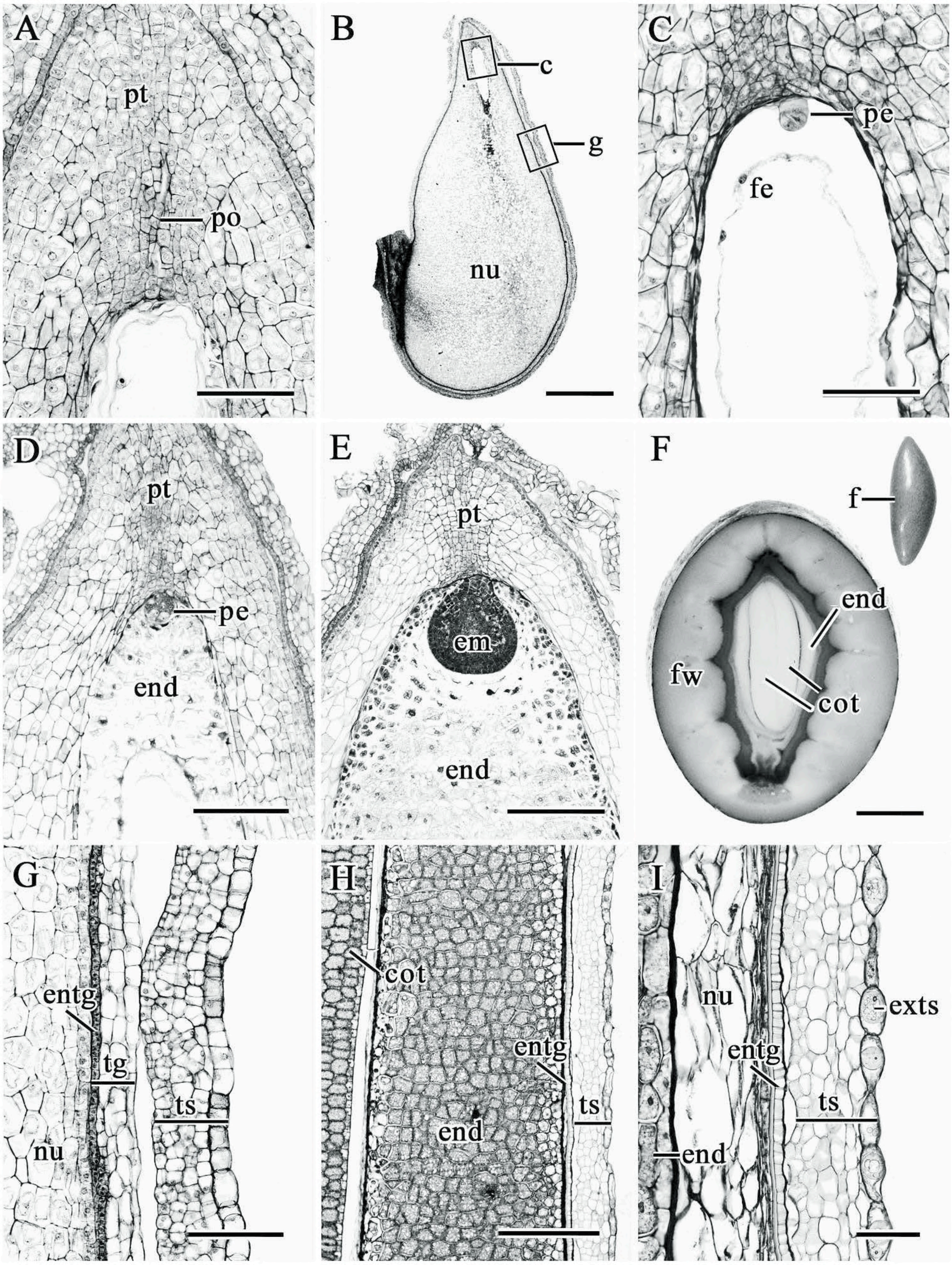

Figure 4. *Leitneria floridana*, development of seeds and seed coats. —A. Longitudinal section of a young seed. —B. Longitudinal section of an entire young seed, showing a thick nucellar tissue. —C. Magnified view of the young seed portion enclosed by rectangle to left of c in B, showing free endosperm nuclei. —D. Longitudinal section of a young seed showing a proembryo and a cellularized endosperm. —E. Longitudinal section of an embryo. —F. Transverse view of a mature fruit cut in upper and lower halves. Lateral view of the intact mature fruit shown at f in upper right. —G. Magnified view of the young seed enclosed by rectangle g in B, showing the structure of a young seed coat. —H. Part of a mature seed transverse section. —I. Part of a mature seed in longitudinal section. Abbreviations: cot, cotyledon; em, embryo; end, endosperm; entg, endotegmen; exts, exotesta; f, fruit; fe, free endosperm nucleus; fw, fruit wall; nu, nucellus; pe, proembryo; po, pollen tube; pt, parietal tissue; tg, tegmen; ts, testa. Scale bars: F = 2 mm; B = 1 mm; D, E, H = 200 μm; A, C, G = 100 μm; I = 50 μm. A–I taken from *D'Arcy, Porter & Demeree 4557* (MO).

Table 1. Comparisons in embryological characters among *Leitneria* Chapm., Simaroubaceae (except for *Leitneria*), Meliaceae, Rutaceae, and Sapindaceae. Data of the Simaroubaceae (except for *Leitneria*), Meliaceae, Rutaceae, and Sapindaceae are based on six, 18, 35, and 20 genera, respectively, whose embryological information is available (see text for all generic names).

	Leitneria	Simaroubaceae	Meliaceae[4]	Rutaceae[9]	Sapindaceae
Anthers and microspores					
Number of sporangia	4	4	4	4	4
Oil gland in anther tissue	absent	absent	absent	scattered	absent
Anther wall formation	?	?	?	?	?
Thickness of anther wall	5 to 6 cells thick	5 to 6 cells thick	5 to 6 cells thick	6 to 7 cells thick	4 to 5 cells thick
Anther epidermis	persistent	persistent	persistent, often papillate	flattened	persistent, flattened
Endothecium	fibrous	fibrous	fibrous	fibrous	fibrous
Middle layers	2 to 3 cells thick, crushed	3 to 4 cells thick, crushed	3 to 4 cells thick, crushed	3 to 4 cells thick, crushed	1 to 2 cells thick
Tapetum	glandular	glandular	glandular	glandular	glandular
Number of nuclei in a tapetal cell	3 to 5; fusing to form polyploid mass	3, 4, 6 to 12; fusing to form polyploid mass	2 to 4(10); fusing to form polyploid mass	2, 3, 4; fusing to form polyploid mass	1, 2, 3; fusing to form polyploid mass
Cytokinesis in meiosis	?	simultaneous	simultaneous	simultaneous	simultaneous
Predominant arrangement of microspore tetrads	tetrahedral	tetrahedral	tetrahedral	tetrahedral, decussate	tetrahedral
Number of cells in a mature pollen	2	2	2, 3	2	2
Anther dehiscence	longitudinal slits*	longitudinal slits	longitudinal slits	longitudinal slits	longitudinal slits
Ovule, nucellus, and megagametophyte					
Ovule orientation	hemitropous	anatropous or hemitropous	anatropous	anatropous (campylotropous in *Cneorum* L.)	ana-, hemi-, campylo-, or amphitropous
Number of archesporial cells	?	2- to 4-celled	1- or multicelled[5]	2- to 5-celled	1(to 4)-celled
Nature of nucellus	crassinucellate	crassinucellate	crassinucellate	crassinucellate	crassinucellate
Thickness of parietal tissue	10 to 15 cells thick	6 to 7 cells thick	6 to 8 cells thick	2 to 4 cells thick	4 to 15 cells thick
Shape of megagaspore tetrad	linear*	linear	linear	linear	linear
Mode of female gametophyte formation	Polygonum type	Polygonum type	Polygonum type	Polygonum type	Polygonum type
Shape of mature female gametophyte	ellipsoidal	obovoid	obovoid	ellipsoidal	narrowly ellipsoidal to lanceoloidal; somewhat curved
Antipodal cells	ephemeral	ephemeral	ephemeral	ephemeral	ephemeral (except in *Cardiospermum* L.)[13]
Nucellar cap	formed, 3-cell-layered	?	formed, 3- to 5-cell-layered or 7- to 9-cell-layered	formed	occasionally formed, 8- to 10-cell-layered (*Acer* L.)
Nucellar tissue in mature ovule	thick	thick	thick	thick	thick or lacking[14]
Specialization of chalaza	unspecialized	unspecialized (*Brucea* J. F. Mill., *Picrasma* Blume) or pachychalazal (*Quassia* L.)	usually pachychalazal[6]	unspecialized (rarely pachychalazal)[10]	enlarged to unspecialized[15]
Hypostase in ovule or young seed	not formed	formed	formed	formed	formed
Obturator	unspecialized	?	formed	funicular, hairy	funicular

Table 1. Continued.

	Leitneria	Simaroubaceae	Meliaceae[4]	Rutaceae[9]	Sapindaceae
Integuments					
Number of integuments	2*	2	2	2	2
Thickness of ii (early stage)	2 cells thick	?	2 cells thick	3 to 4 cells thick	?
Thickness of ii (late stage)	3 to 4 cells thick	2 to 6 cells thick	2 to 4 cells thick	3 to 4 cells thick	4 to 8 cells thick
Inner integument multiplicative?	no	no	no	no	slightly yes
Thickness of oi (early stage)	2 cells thick	?	2 cells thick	4 to 5 cells thick	?
Thickness of oi (late stage)	3 cells thick	3 to 10 cells thick	2 to 4 cells thick	4 to 5 cells thick	8 to 70 cells thick
Outer integument multiplicative?	no	usually no	no	no	yes
Vascular bundles in ii (or tegmen)	absent	absent	absent	absent	absent
Vascular bundles in oi (or testa)	absent	absent	absent	absent	present
Micropyle formation	ii	ii	ii, rarely ii and oi[7]	ii and oi	ii, or ii and oi
Micropyle form	ii, extremely elongated; irregularly folded	ii, extremely elongated, irregularly folded (*Brucea*); unspecialized (*Quassia*)[1]	unspecialized	unspecialized	unspecialized
Endothelium	not formed	not formed	not formed	not formed	not formed
Fertilization, endosperm, and embryo					
Path of pollen tube	probably porogamous	porogamous	porogamous	porogamous	porogamous
Mode of endosperm formation	nuclear	nuclear	nuclear	nuclear	nuclear
Type of embryogeny	?	Onagrad (*Brucea amarissima* (Lour.) Mill.)	?	Asterad, Onagrad, Solanad[11]	Asterad
Suspensor	not pronounced	not pronounced	not pronounced	not pronounced	not pronounced
Mature seed					
Shape or curvature	straight	straight	straight, often large	straight (curved in *Cneorum*)	curved
Aril or arilloid	absent	absent	often present (sometimes as a wing)[8]	rarely present (*Eriostemon* Sm., *Pilocarpus* Vahl, etc.)	present
Endosperm in mature seed	present, 10 to 30 cells thick	present, 6 to 7 cells thick[2]	present (absent in *Trichilia* P. Browne)	present or absent[12]	absent
Perisperm	absent	present, 3 to 4 cells thick	absent	absent	absent
Shape of embryo in mature seed	straight	straight	straight	straight (curved in *Cneorum*)	curved
Mature seed coat					
Seed coat type	testal	testal[3]	usually testal	usually exotestal or mesotestal	exotestal
Testa multiplicative?	yes (but weakly)	yes (but weakly)	usually yes	usually yes	yes
Thickness of testa	5 to 11 cells thick	3 to 10 cells thick	2 to 40 cells thick	4 to 21 cells thick	8 to 70 cells thick

Table 1. Continued.

	Leitneria	Simaroubaceae	Meliaceae[4]	Rutaceae[9]	Sapindaceae
Cells of exotesta	more or less enlarged, containing granular pigments	unspecialized (*Quassia*) or tubular (*Picrasma*, *Simarouba* Aubl.); walls slightly lignified (*Brucea*)	unspecialized, cuboidal, or palisadal; thin- or thick-walled, pitted	radially elongate (thus palisadal) in groups or evenly, longitudinally elongate, or cuboidal; walls thin or thick (sometimes fibrous); sometimes mucilaginous	typically palisadal, sometimes stellate-prismatic or flattened, rarely hairy
Cells of mesotesta	unspecialized	unspecialized or aerenchymatous; idioblasts scattered (*Simarouba*)	unspecialized and thin-walled when present; often collapsing or crushed	thin- or thick-walled, sometimes differentiating into an outer and an inner layer; cells of outer layer sometimes containing brown contents, and those of inner layer sometimes crushed or persistent with a crystal	unspecialized, a thick tissue composed of a compact outer half and an aerenchymatous inner half
Cells of endotesta	unspecialized	unspecialized (*Brucea*, *Quassia*), slightly lignified on radial and inner walls (*Picrasma*), or cuboidal with lignified pitted thickenings (*Simarouba*)	unspecialized, small, with 1 or 2 crystals	unspecialized or crystalliferous	unspecialized
Tegmen multiplicative?	no	no	usually no	no	usually no
Thickness of tegmen	1 cell thick*	crushed (*Picrasma*) or 1 cell thick (*Brucea*)	usually 4 to 7 cells thick, rarely 30 cells thick (*Carapa guianensis* Aubl.)	NA	3 to 12 cells thick
Cells of exotegmen	crushed*	crushed	usually fibrous	crushed	usually unspecialized
Cells of mesotegmen	crushed*	crushed	usually crushed; outer layers rarely persisting as elongate sclerotic cells	crushed	unspecialized
Cells of endotegmen	slightly thickened; containing darkly staining contents early in development	usually crushed, but persistent in *Brucea amarissima* with cells filled with darkly staining contents	unspecialized, small	crushed, rarely persistent, tanniniferous	unspecialized

Table 1. Continued.

	Leitneria	Simaroubaceae	Meliaceae[4]	Rutaceae[9]	Sapindaceae
References	Pfeiffer (1912), this study	Corner (1976), Nair & Joseph (1957), Nair & Sukumaran (1960), Narayana (1957), Wiger (1935)	Boesewinkel (1981), Corner (1976), Garudamma (1956, 1957), Ghosh (1966a, 1966b), Nair (1958, 1959a, 1959b), Narayana (1958), Prakash et al. (1977), Wiger (1935)	Bacchi (1943), Banerji (1954), Boesewinkel (1977, 1978, 1984), Boesewinkel & Bouman (1978), Corner (1976), Desai (1962), Johri & Ahuja (1957), Mauritzon (1935, 1936), Narayana (1963)	Banerji & Chaudhuri (1944), Corner (1976), David (1938), Guérin (1901), Haskell & Postlethwait (1971), Khushalani (1963), List & Steward (1965), Mauritzon (1936), Nair & Joseph (1960), Netolitzky (1926), Tobe & Peng (1990), van der Pijl (1957)

Abbreviations: ii, inner integument; oi, outer integument; NA, not applicable.

* Data of Pfeiffer (1912) were confirmed.

[1] This character has been reported by Wiger (1935) from *Ailanthus altissima* (Mill.) Swingle, *A. glandulosa* Desf., *A. malabarica* DC., *Brucea amarissima*, *Quassia amara* L., *Samadera indica* Gaertn., *S. mekongensis* Engl.; by Nair and Joseph (1957) from *S. indica*; by Nair and Sukumaran (1960) from *B. amarissima*; by Narayana (1957) from *A. excelsa* Roxb.; by Corner (1976) from *B. javanica* (L.) Merr. and *Q. indica* (Gaertn.) Noot.

[2] Endosperm is lacking in mature seeds in *Picrasma*, 6 to 7 cells thick in *Brucea* (Corner, 1976).

[3] Although the testa is unspecialized, its cells are persistent except in *Brucea javanica*, where they also degenerate (Corner, 1976).

[4] Data for *Chisocheton* Blume are excluded because the genus is obviously at a derived position in Meliaceae phylogeny (Muellner et al., 2003) and exhibits distinct features such as the unitegmy and orthotropy of ovule and the vascularized integument.

[5] It is reported that the ovule archesporium is 1-celled but sometimes 2- to 3-celled in *Naregamia alata* Wight & Arn. (Nair, 1959a) and *Cipadessa baccifera* (Roth) Miq. (Narayana, 1958), while it is multicelled, for instance, 3- to 4-celled in *Sandoricum indicum* Cav. (Nair, 1958), 6- to 8-celled in *Azadirachta indica* A. Juss. (Nair & Kanta, 1961), or up to 11-celled in *Lansium domesticum* Corrêa (Prakash et al., 1977).

[6] Pachychalazal or perichalazal seeds occur in many genera of the Melioideae and Cedreloideae (*Aglaia* Lour., *Aphanamixis* Blume, *Carapa* Aubl., *Dysoxylum* Blume, *Lansium* Corrêa, *Swietenia* Jacq., and *Trichilia*); seeds with these enlarged chalazae are rare in the Melioideae (*Melia azedarach* L.) and Cedreloideae (*Cedrela toona* Rottler).

[7] The micropyle is formed by the inner integument in *Naregamia alata* (Nair, 1959a), *Melia azedarach* (Nair, 1959b), *Sandoricum indicum* (Nair, 1958), *Azadirachta indica* (Garudamma, 1957; Nair & Kanta, 1961), and *Cipadessa baccifera* (Narayana, 1958), but it is formed by both the inner and outer integuments in *Aphanamixis grandiflora* Blume (Wiger, 1935) and *Lansium domesticum* (Prakash et al., 1977).

[8] Arillate seeds occur in some genera (*Aglaia*, *Aphanamixis*, *Dysoxylum*, and *Lansium*) of the Melioideae; winged seeds occur in the Cedreloideae (*Cedrela* P. Browne and *Swietenia*). Seeds of some genera (*Guarea* F. Allam. ex L., *Melia* L., and *Trichilia*) of Melioideae do not have appendages.

[9] Data for *Glycosmis* cf. *arborea* (Roxb.) DC. are excluded because the genus is obviously at a derived position in Rutaceae phylogeny (Muellner et al., 2003) and exhibits distinct features such as the unitegmy of ovule and the vascularized integument (Boesewinkel & Bouman, 1978).

[10] According to Corner (1976), seeds have unspecialized chalaza in most genera (*Calodendrum* Thunb., *Chloroxylon* DC., *Dictamnus* L., *Pilocarpus*, *Ruta* L., and *Zanthoxylum* L.) but are clearly pachychalazal in *Atalantia monophylla* (L.) DC. The pachychalazal seeds also occur in *Glycosmis* cf. *arborea* (Boesewinkel & Bouman, 1978).

[11] The Asterad type is reported in *Ravenia spectabilis* (Lindl.) Engl., Onagrad type in *Ruta graveolens* L., and Solanad type in *Citrus trifoliata* L.

[12] According to Corner (1976), seeds are albuminous in *Dictamnus albus* L. and *Ruta graveolens* but are exalbuminous in *Atalantia monophylla* and *Pilocarpus racemosus* Vahl.

[13] In *Cardiospermum halicacabum* L. the antipodal cells are usually more than three and as many as 14 cells are observed. They may be multinucleate and persist even after fertilization up to the 3- or 4-celled proembryo stage and degenerate afterward (Nair & Joseph, 1960).

[14] In *Cardiospermum halicacabum* the nucellar tissue is consumed by an enlarging female gametophyte except at the apical part (Nair & Joseph, 1960).

[15] According to Corner (1976), the chalaza of seed is unspecialized in *Alectryon* Gaertn. and *Dodonaea* Mill., distinctly enlarged in *Guioa* Cav. and *Sapindus* L., elongate transversely in *Nephelium* L., or develops into an extensively pachychalazal structure in *Allophylus* L., *Cardiospermum*, *Cupania* L., *Harpullia* Roxb., and *Paullinia* L.

nucellar cap; the hypostase is not differentiated, and the obturator is absent.

The ovule is bitegmic, with both the inner and outer integuments initially two cell layers thick, soon thickening to three (or four) cell layers. The inner and outer integuments lack vascular bundles, and the outer integument is always shorter than the inner. The micropyle is formed by the inner integument alone; the inner integument is extremely elongate to beyond the apex of the nucellus, and is irregularly folded.

Fertilization is probably porogamous in *Leitneria floridana*, with nuclear-type endosperm formation. The mature seeds are albuminous, with a 10- to 30-cell-layered endosperm. Embryogenesis is uncertain, and the mature seed embryo is straight, large, and dicotyledonous.

The mature seed is straight and exarillate; the mature seed coat is testal, comprising a 5- to 11-cell-layered testa and a 1-cell-layered tegmen (endotegmen); the other cell layers of the tegmen are crushed. Cells of the exotesta are slightly enlarged, containing granular pigments; cells of the mesotesta and endotesta are unspecialized, while those of the endotegmen are slightly thick-walled.

COMPARISONS WITH OTHER SIMAROUBACEAE AND RELATED FAMILIES

I compared the embryological features of *Leitneria* with those from a wide range of members of the Simaroubaceae (19 genera), Meliaceae (50 genera), and Rutaceae (161 genera), using the family Sapindaceae s.l. (including Aceraceae and Hippocastanaceae) as an outgroup. None of these families have been subjected to comprehensive embryological examination, and even for species investigated thus far, data are as yet fragmentary. Embryological data for the Simaroubaceae have been collected from 12 species of the genera *Ailanthus*, *Brucea*, *Picrasma*, *Quassia* L., *Samadera* Gaertn., and *Simarouba* Aubl. (Wiger, 1935; Nair & Joseph, 1957; Narayana, 1957; Nair & Sukumaran, 1960; Corner, 1976); those for the Meliaceae are from 18 genera in two major clades representing the subfamilies Cedreloideae (*Carapa* Aubl., *Cedrela* P. Browne, *Chukrasia* A. Juss., *Khaya* A. Juss., and *Swietenia* Jacq.) and Melioideae (e.g., *Aglaia* Lour., *Aphanamixis* Blume, *Chisocheton* Blume, *Cipadessa* Blume, *Dysoxylum* Blume, *Guarea* F. Allam. ex L., *Lansium* Corrêa, *Melia* L., *Naregamia* Wight & Arn., *Sandoricum* Cav., *Trichilia* P. Browne, *Turraea* L., and *Walsura* Roxb.) (Wiger, 1935; Garudamma, 1956, 1957; Nair, 1958, 1959a, 1959b; Narayana, 1958; Nair & Kanta, 1961; Ghosh, 1966a, 1966b; Corner, 1976; Prakash et al., 1977; Boesewinkel, 1981) within the family phylogeny (Muellner et al., 2003); those for the Rutaceae are from 35 genera (*Adenandra* Willd., *Aegle* Corrêa, *Agathosma* Willd., *Atalantia* Corrêa, *Boenninghausenia* Rchb. ex Meisn., *Boronia* Sm., *Calodendrum* Thunb., *Chloroxylon* DC., *Choisya* Kunth, *Citropsis* (Engl.) Swingle & M. Kellerm., *Citrus* L., *Clausena* Burm. f., *Cneorum* L., *Coleonema* Bartl. & H. L. Wendl., *Dictamnus* L., *Dictyoloma* A. Juss., *Diosma* L., *Empleurum* Aiton, *Eriostemon* Sm., *Erythrochiton* Nees & Mart., *Flindersia* R. Brown, *Fortunella* Swingle, *Glycosmis* Corrêa, *Limonia* L., *Melicope* J. R. Forst. & G. Forst., *Murraya* Köenig ex L., *Phellodendron* Rupr., *Pilocarpus* Vahl, *Poncirus* Raf., *Ptelea* L., *Ruta* L., *Skimmia* Thunb., *Spathelia* L., *Triphasia* Lour., and *Zanthoxylum* L.) (Mauritzon, 1935, 1936; Bacchi, 1943; Banerji, 1954; Johri & Ahuja, 1957; Desai, 1962; Narayana, 1963; Corner, 1976; Boesewinkel, 1977, 1978, 1984; Boesewinkel & Bouman, 1978); those for the Sapindaceae are from 20 genera (*Acer* L., *Aesculus* L., *Alectryon* Gaertn., *Allophylus* L., *Cardiospermum* L., *Cupania* L., *Diplopeltis* Endl., *Dodonaea* Mill., *Guioa* Cav., *Harpullia* Roxb., *Koelreuteria* Laxm., *Litchi* Sonn., *Magonia* A. St.-Hil., *Nephelium* L., *Paullinia* L., *Pometia* J. R. Forst. & G. Forst., *Sapindus* L., *Trigonachras* Radlk., *Ungnadia* Endl., and *Xanthoceras* Bunge) (Guérin, 1901; Netolitzky, 1926; Mauritzon, 1936; David, 1938; Banerji & Chaudhuri, 1944; van der Pijl, 1957; Nair & Joseph, 1960; Khushalani, 1963; List & Steward, 1965; Haskell & Postlethwait, 1971; Corner, 1976; Tobe & Peng, 1990). The Rutaceae and Sapindaceae, which are embryologically relatively well studied, have diverse features, particularly in seed and seed coat characters (Table 1). Thus, I collected embryological data for the Rutaceae from *Cneorum* (Boesewinkel, 1984), which represents the basal lineage within the family phylogeny (Muellner et al., 2007); data for the Sapindaceae were collected from *Xanthoceras* of the Xanthoceroideae (Guérin, 1901; Corner, 1976), which likely represents the first lineage to diverge within the entire family (Harrington et al., 2005).

Comparisons show that *Leitneria* has many embryological features in common with the Simaroubaceae, Meliaceae, Rutaceae, and Sapindaceae. Even features that appeared distinctive to *Leitneria* and were expected to be of use in critical comparisons (e.g., multinucleate anther-tapetal cells, thick nucellar cap) occurred widely in all of the families compared. Such shared features do not provide evidence of affinities between *Leitneria* and any particular family or limited group of families. However, *Leitneria* either shared or

Table 2. Embryological comparisons among *Leitneria* Chapm., Simaroubaceae (excluding *Leitneria*), Meliaceae, and Rutaceae (*Cneorum* L.). Character-state polarities were determined using the Sapindaceae (*Xanthoceras* Bunge) as an outgroup. Plesiomorphy is scored 0, and apomorphy 1. Data of the Simaroubaceae (except for *Leitneria*) and Meliaceae are based on six and 18 genera, respectively, whose embryological information is available (see text for all generic names). The numbers (1 to 11) that precede the characters correspond to the numbers appearing on the phylogenetic diagram in Figure 5.

Character	*Leitneria*	Simaroubaceae	Meliaceae	Rutaceae (*Cneorum*)	Sapindaceae (*Xanthoceras*)
1. Specialization of chalaza	unspecialized (0)	unspecialized (0)	pachychalazal or perichalazal (1)	unspecialized (0)	unspecialized
2. Seed shape or curvature	straight (1)	straight (1)	straight (1)	campylotropous (0)	campylotropous
3. Appendage (aril or wing) on seed	absent (0)	absent (0)	present (1)	absent (0)	absent
4. Testa multiplicative	no (1)	no (1)	yes (0)	no (1)	yes
5. Vascular bundles in testa	absent (1)	absent (1)	absent (1)	absent (1)	present
6. Cells of mesotesta	unspecialized (1)	unspecialized (1)	unspecialized (1)	unspecialized (1)	thick-walled
7. Endotesta crystalliferous	no (0)	no (0)	yes (1)	no (0)	no
8. Tegmen multiplicative	no (1)	no (1)	no (1)	no (1)	yes
9. Exotegmen fibrous when present	no (0)	no (0)	yes (1)	no (0)	no
10. Tip of inner integument forming a micropyle	elongated, irregularly folded (1)	elongated, irregularly folded (*Brucea* J. F. Mill.) (1); unspecialized (*Quassia*) (0)	unspecialized (0)	unspecialized (0)	unspecialized
11. Mature tegmen composed only of endotegmen	yes (1)	yes (*Brucea amarissima* (Lour.) Merr.) (1); no (0)	no (0)	no (0)	no

did not share 11 mature ovule or seed structures with all or some of the families compared. The respective character states in *Leitneria* and the related families are summarized in Table 2, and the distribution of apomorphies in nine characters is mapped on the phylogenetic diagram in Figure 5.

Leitneria and the families Simaroubaceae, Meliaceae, and Rutaceae all have a non-vascularized testa, an unspecialized mesotesta, and a non-multiplicative tegmen (see characters 5, 6, and 8 in Table 2); the family Sapindaceae (*Xanthoceras*) has vascular bundles in the testa (which develops into a persistent tissue composed of thick-walled cells) and a multiplicative tegmen that develops into a thick, permanent tissue. These shared features are likely to be synapomorphies, corroborating known close molecular relationships among the Simaroubaceae, Meliaceae, and Rutaceae (Fernando et al., 1995; Gadek et al., 1996; Muellner et al., 2007). Among these families, the Meliaceae are characterized by pachychalazal or perichalazal seeds, arillate or winged seeds, a crystalliferous endotesta, and a fibrous exotegmen (characters 1, 3, 7, and 9). In contrast to the Meliaceae, which have family-specific characteristics, the Simaroubaceae and Rutaceae appear to have no autapomorphies. The Simaroubaceae have straight seeds in common with the Meliaceae (character 2) and a non-multiplicative

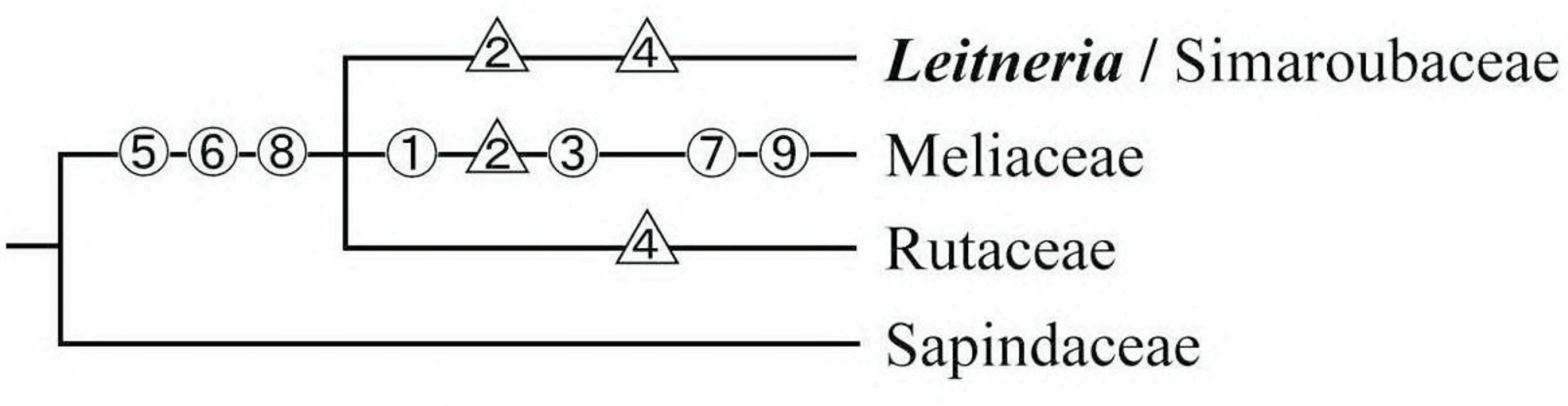

Figure 5. Phylogenetic diagram (modified from Muellner et al., 2007) for *Leitneria*, Simaroubaceae (excluding *Leitneria*), Meliaceae, Rutaceae, and Sapindaceae (outgroup), showing the distribution of apomorphies in Table 2. Numbers correspond to character states described in Table 2; a character enclosed in a circle indicates a synapomorphy or autapomorphy, while a triangle denotes a synapomorphy or homoplasy.

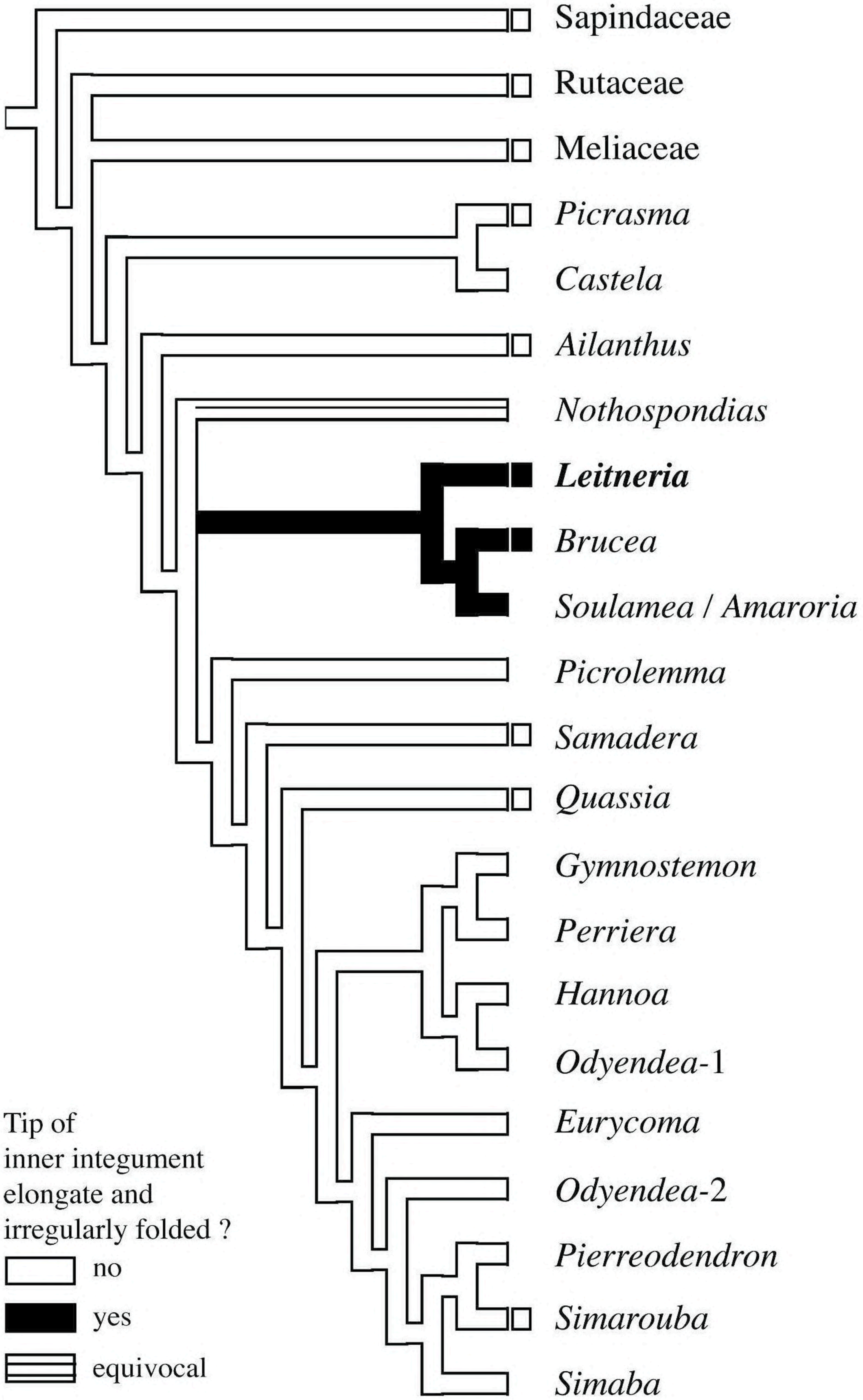

Figure 6. Taxonomic distribution of micropyles formed by elongated, irregularly folded inner integuments within the phylogenetic tree for the Simaroubaceae (modified from Clayton et al., 2007).

testa in common with the Rutaceae (character 4). All of these seed and seed coat features are found in *Leitneria*. Thus, although an understanding of simaroubaceous embryological features is still incomplete, *Leitneria* apparently fits best within the family Simaroubaceae.

COMPARISONS BETWEEN *LEITNERIA* AND OTHER SIMAROUBACEAE

Among simaroubaceous genera for which embryological data are available, *Brucea* has the closest resemblance to *Leitneria* in that both genera form a micropyle from an elongate, irregularly folded inner integument (see character 10 in Table 2). In addition to the two species of *Brucea*, micropyle formation has been examined in eight species of the genera *Ailanthus*, *Quassia*, and *Samadera* (see footnote 1 in Table 1). Wiger (1935: 83), who examined six species of *Ailanthus*, *Quassia*, and *Samadera* in addition to *B. amarissima* (Lour.) Merr., noted that "The further development in *Brucea* is rather interesting. The inner integument is tube-like and lengthened and by pushing against the placenta more or less folded, the micropyle thus being rather long and winding." Nair and Sukumaran (1960), who also studied ovule development in *B. amarissima*, did not examine the micropyle, but a drawing of the mature ovule (Nair & Sukumaran, 1960: 176, fig. 28) clearly demonstrates that the micropyle is formed by an elongate, irregularly folded inner integument. A similar micropyle structure is also found in *B. javanica* (L.) Merr. (Corner, 1976: 459, fig. 536); Corner (1976: 253) reported that in *B. javanica* the inner integument elongates beyond the exostome and is often splayed out against the carpel wall. As noted above, a molecular sister-group relationship exists between *Leitneria* and the clade comprising *Brucea*, *Soulamea*, and *Amaroria* (Clayton et al., 2007). When mapped on the phylogenetic tree of the Simaroubaceae with MacClade vers. 3.04 software (Maddison & Maddison, 2005), micropyles formed from an elongate, irregularly folded inner integument were restricted to *Leitneria* and the clade containing *Brucea*, *Soulamea*, and *Amaroria* (Fig. 6). Thus, a morphological synapomorphy very likely unites *Leitneria* and the clade comprising *Brucea*, *Soulamea*, and *Amaroria* (Fig. 6). We have no information on the micropyles of *Soulamea* and *Amaroria*, but they may share with *Leitneria* and *Brucea* a micropyle formed by an elongate, irregularly folded inner integument. The significance of this type of micropyle formation is not clear, but it may play a role in selecting pollen for fertilization, particularly in the anemophilous *Leitneria*.

Seed coat structure may provide yet another synapomorphy for the clade containing *Leitneria*, *Brucea*, *Soulamea*, and *Amaroria*. In *Leitneria* the mature seed coat is composed of a testa with five to 11 cell layers and a tegmen of just one cell layer (i.e., an endotegmen). While the cells of the testa are little specialized, those of the endotegmen, which is the only persistent cell layer in the tegmen (character 11 in Table 2), stained darkly early in development and were somewhat thick-walled at maturity. According to Nair and Sukumaran (1960), cells of the endotegmen in *B. amarissima* are persistent and have thick walls and a dense accumulation of darkly staining contents. Thus, the seed coat structure of *Leitneria* is very similar to that of *B. amarissima*. Corner (1976) reported that all cells of the tegmen are crushed in *B. javanica*, *Picrasma javanica* Blume, *Quassia indica* (Gaertn.) Noot., and *Simarouba* spp., but this needs confirmation because observations on *B. amarissima* (Nair & Sukumaran, 1960) and *B. javanica* (Corner, 1976) are not concordant.

Leitneria has a very thick parietal tissue in the nucellus that persists until late in the post-fertilization stages. In other angiosperms, the parietal tissue is generally destroyed by an enlarging female gametophyte before and after fertilization. The persistent thick parietal nucellar tissue of *Leitneria* may provide another key character for future comparisons with other Simaroubaceae.

In conclusion, although previous morphology-based systematic treatments have not suggested a simaroubaceous affinity for *Leitneria*, my embryological studies showed that features of the genus are concordant with members of the Simaroubaceae, particularly *Brucea*, corroborating molecular evidence (Clayton et al., 2007). Extensive studies on the Simaroubaceae (particularly genera considered basal [e.g., *Ailanthus*, *Castela* Turpin, and *Picrasma*] in the family phylogeny) would provide invaluable evidence for further assessment of the systematic position of *Leitneria*.

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